Development of a Technique for Analyzing 15N in Waters With Low Nitrate Content

DEVELOPMENT OF A TECHNIQUE FOR ANALYZING ^{15}N IN WATERS WITH LOW NITRATE CONTENT

Final Report

Submitted by

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INTRODUCTION

The Earth's atmosphere, which contains approximately 75% of all nitrogen, is comprised of 78.03 % nitrogen, 20.99% oxygen, and increasingly smaller traces of argon, carbon dioxide, neon, helium, krypton, and xenon (Chang, 1994). While the remaining 25% of the total available nitrogen is mostly locked in crustal rocks a small percentage is also found in both the hydrosphere and biosphere (Hem, 1992). With so much of the atmosphere being comprised of dinitrogen gas (N_2), this elemental form of nitrogen is essentially unavailable for use by biological organisms.

In addition to N_2, nitrogen is also present as organic nitrogen (in plants, animals, microbial biomass, and soil organic matter) and inorganic nitrogen, as ammonia and nitrate ions (Myrold, 1999). Because nitrogen is a core constituent of protein, one of the major building blocks in all living material, it is a fundamental nutrient required by all living organisms (Smith, 1992). It is also the one nutrient that most often limits plant growth in terrestrial ecosystems (Myrold, 1999).

In a process called fixation, dinitrogen gas is initially transformed into more useable forms of nitrogen in one of two ways: A small amount is naturally combined with the oxygen and hydrogen in water through high-energy fixation by means of cosmic radiation, meteorite trails, or lightening resulting in ions of ammonia and nitrate {as nitric acid (H_2NO_3)} being carried to earth in rainwater (Smith, 1992). A far greater amount of

nitrogen, however, is fixed as ammonia biologically. At amounts 10 - 20 times higher than that fixed through high-energy fixation, approximately 90% of the amount of nitrogen fixed is accomplished by various symbiotic bacteria, free-living aerobic bacteria, and blue-green algae (Smith, 1992). Nitrogen is fixed when these organisms split molecular nitrogen (N_2) into two atoms of N. The free N atoms then combine with hydrogen to form two molecules of ammonia (Smith, 1992).

Ammonia ions, or ammonium (NH_4^+), are also created through a process called ammonification. This is the conversion of organic-nitrogen compounds to ammonium by enzymes produced by microbes and soil animals (Myrold, 1999). As ammonium is absorbed by plants through their root systems and incorporated into amino acids it is passed through the food chain. These amino acids are subsequently broken down by heterotrophic bacteria and fungi when they decompose wastes and dead animal and plant tissues to obtain carbon for growth and cell synthesis (Smith, 1992). This process releases inorganic nitrogen in the form of ammonium and nitrate back into the ecosystem.

When nitrogen is a limiting factor in this process microbes will retain the ammonium within their cells. The ammonium becomes immobilized in the biomass and is unavailable for other uses until it, in turn, becomes available as a part of the decomposition process (Sylvia *et al.*, 1999). If the amount of nitrogen available is not a limiting factor, an abundance of ammonium is produced whereby other bacteria are able to consume it (Sylvia *et al.*, 1999).

Through the process of nitrification, specialized bacteria oxidize ammonium to produce nitrite;

$$2NH_4^+ + 3O_2 \text{ —(}\textit{Nitrosomonas}\text{ bacteria)—> } 2NO_2^- + 4H^+ + 2H_2O + \text{Energy}$$

and then nitrate;

$$2NO_2^- + O_2 \text{ —(}\textit{Nitrobacter}\text{ bacteria)—> } 2NO_3^- + \text{Energy}$$

which is the inorganic form of nitrogen that is most essential for vegetative growth (Miller and Donahue, 1995). Through this process of nitrification, *Nitrosomonas* bacteria utilize ammonium as an energy source in the transformation of ammonium to nitrite. The nitrite formed through this oxidation process provides an energy source for *Nitrobacter* bacteria in the transformation of nitrite to nitrate (Miller and Donahue, 1995).

Nitrates may be utilized by plants, immobilized by microbes, stored in decomposing humus, or leached away into surface or ground water supplies (Smith, 1992). While both ammonium and nitrate ions are very soluble in water, the positively charged ammonium ion is held to cation exchange sites and resists leaching (Miller and Donahue, 1995). Nitrate that leaches eventually enters ground and surface waters where it has the potential to create such adverse environmental effects as eutrophication (Sylvia *et al.*, 1999).

The transformation of dinitrogen gas and organic nitrogen into inorganic forms occurs as a result of oxidation processes under aerobic conditions (Smith, 1992). The resulting nitrate in turn provides the necessary substrate for the reduction process of denitrification. Denitrifiers, dominated by the bacteria genus *Pseudomonas*, convert nitrate back to gaseous forms such as nitrous oxide (N_2O), nitric oxide (NO), and nitrogen dioxide (NO_2) referred to collectively as nitrogen oxides or NO_x (Smith, 1992). Denitrification is probably the most extensive gaseous loss of nitrogen. Under anaerobic conditions there are also a few specifically adapted bacteria that use nitrogen, as nitrate, as an electron

acceptor (Miller and Donahue, 1995). This results in losses of nitrogen as dinitrogen gas or nitrous oxide.

The primary source of nitrogen in pristine alpine watersheds is atmospheric deposition because the headwater location itself precludes inputs of groundwater from outside the drainage basin.(Campbell *et al.*, 1995). These high-altitude ecosystems tend to be highly sensitive to changes in the flux of energy, chemicals, and water because they have extensive areas of exposed and unreactive bedrock, poorly developed soils with limited vegetation, and short growing seasons (Baron , 1991; Walker *et al.*, 1994). In these natural environments, the availability of nitrogen is often limited by the rate of nitrogen-fixation occurring within the system (Silva *et al.*, 2000). Forested ecosystems, which are generally nitrogen-limited, are characterized as having efficient internal nitrogen cycling capabilities. This results in minimal losses of inorganic nitrogen in surface waters, groundwater, and gaseous loss through denitrification (Tamm, 1991).

In many upland catchments increased nitrate concentrations can be found in stream water during early snowmelt periods (Kendall *et al.*, 1995). It is likely that this is a result of both atmospheric deposition of nitrate and ammonium in the snowpack and soil-derived nitrate (Kendall *et al.*, 1995). In these high elevation basins, it has been found that a significant fraction of the atmospheric nitrogen deposition is stored in seasonal snowpacks and released in an ionic pulse during the first portion of snowmelt (Williams & Melack, 1991a). Recent work has suggested that most of this snowmelt enters the soil and has the potential to interact with both inorganic and organic soil nitrogen pools (Williams & Melack, 1991b; Williams *et al.*, 1995). Much of the nitrate found in these surface waters

appears to be nitrogen from atmospheric deposition that has been assimilated and mineralized over time periods of several months, up to a year (Kendall *et al.*, 1995). Because many of these high-altitude areas tend to have less developed soil structure and/or vegetative cover, it is possible they may have less time for assimilation and therefore export greater amounts of nitrate from the subsurface (Williams *et al.*, 1996).

The hydrologic cycle of temperate high-altitude watersheds is characterized by a snowpack accumulation period during late autumn, winter, and early spring; a snowmelt runoff period during late spring and early summer; and a runoff period in late summer and early autumn which is predominantly baseflow (Campbell *et al.*, 1995). Nitrate concentrations in surface waters of high-elevation catchments in the western U.S. show a characteristic increase in nitrate concentrations coincident with the initiation of snowmelt runoff (Williams *et al.*, 1996). With no flushing events occurring throughout autumn and winter to remove accumulated products of decomposition, the soil pore water tends to become highly concentrated in nitrogen before snowmelt begins (Campbell *et al.*, 1995). As a result, streamflow at the beginning of the melt cycle has been found to be dominated by water that was present in the watershed before snowmelt begins and that has been displaced into the stream as snowmelt infiltrates the soil (Pilgrim, 1979; Mast *et al.*, 1995).

While some of the nitrate found in early snowmelt runoff is from atmospheric deposition eluted through the snowpack, evidence shows that nitrate formed within the soil is also an important contributor to surface waters (Peter and Driscoll, 1987; Rascher *et al.*, 1987). In Colorado, over-winter nitrogen mineralization inputs to the soil inorganic nitrogen pool were more than an order of magnitude higher than atmospheric inputs and

appeared to be related to the insulating effect of a continuous snowpack, which allowed microbial activity to begin well before snowmelt (Brooks *et al.*, 1995). It has been shown that as a consistent snowpack begins to accumulate, and snow depths increase, soil temperatures begin to warm. In their Colorado study, a consistent snow cover insulated the soil surface from extreme air temperatures and allowed the soils to warm well before snowmelt began (Brooks *et al.*, 1995).

There is also evidence that nitrification rates are increased by the soil freezing (Edwards and Cresser, 1992). Observations indicate that as soils thaw, first at the surface, and later at lower depths, the freeze/thaw cycle releases labile nitrogen compounds from ruptured cell membranes, providing substrate for microbial activity (Edwards and Cresser, 1992; Brooks *et al.*, 1995). The importance of this microbial activity is evidenced by the measurement of carbon dioxide fluxes under the snowpack (Sommerfeld *et al.*, 1993; Brooks *et al.*, 1996). Another study suggests that recent reports of elevated carbon dioxide fluxes through snow indicate that microbial activity under snow in alpine ecosystems is much greater than previously thought (Williams *et al.*, 1996).

In other studies conducted in Colorado's Rocky Mountains, it has been shown that, even with increasing amounts of nitrate, little ammonium is found in surface waters of high-elevation catchments at any time (Baron, 1991; Stottlemyer and Troendle, 1992). Several researchers suggest that ammonium released from the snowpack is immobilized by microbial activity and/or adsorbed on soil exchange sites as snowmelt infiltrates the soil (Brooks *et al.*, 1995; Williams *et al.*, 1995). Kendall *et al.* (1995) suggest that ammonium stored in the seasonal snowpack may be transformed to nitrate before release in the

the nitrate concentrations found in snowmelt runoff.

Lakes in the Rocky Mountains are relatively uncontaminated compared to many other high-elevation lakes in the world (Psenner, 1989). However, nitrate concentrations in many streams now remain elevated throughout the growing season, indicating that some of these high-elevation catchments have become nitrogen-saturated (Baron *et al.*, 1994; Campbell *et al.*, 1995; Williams *et al.*, 1996a). This may indicate that there has been a shift in ecosystem dynamics from a nitrogen-limited system to a nitrogen-saturated system as a result of anthropogenically fixed nitrogen in wetfall and dryfall (Williams *et al.*, 1996). It has been suggested that the resulting increases in wet and dry deposition of nitrogen are beginning to change the fundamental nitrate-discharge patterns in high-elevation catchments of the western U.S. (Williams *et al.*, 1996).

Using nitrogen's stable isotopes, ^{14}N and ^{15}N, one can more easily determine whether levels of nitrate in runoff are occurring from atmospheric deposition or because of enhanced cycling within the terrestrial ecosystem. The use of these stable isotopes can provide a means of source identification because different sources of nitrate often have isotopically distinct nitrogen compositions. Due to the amount of energy involved in utilizing compounds, organisms will preferentially use a lighter isotope over heavier ones. As a result, almost anything created by an organism will be isotopically lighter than the material not used (Kendall, 1998).

Stable isotopes are nuclides that do not appear to decay to other isotopes on geologic time scales, but may themselves be produced by the decay of radioactive isotopes (Kendall

and Caldwell, 1998). Naturally occurring stable isotopes of elements found in abundance in our environment include those of hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulfur (S).

Environmental isotopes are natural and anthropogenic isotopes whose wide distribution in the hydrosphere can assist in the solution of such hydrological questions as:

- determination of the role of atmospheric deposition in controlling water chemistry
- identification of the sources of solutes in contaminated systems and
- assessment of biologic cycling of nutrients within an ecosystem (Kendall and Caldwell, 1998).

While the average abundance of ^{15}N in air is constant, nitrogen isotopes are generally reported in $^{0}/_{00}$ relative to N_2 in atmospheric air, using the standard definition of δ (delta):

$$\delta^{15}N_{AIR} = \{[(^{15}N/^{14}N)_X / (^{15}N/^{14}N)_{AIR}]-1\} * 1000$$

where x = sample and AIR = the reference standard gas (Kendall, 1998). Isotopic compositions of materials analyzed on Mass Spectrometers are usually reported relative to some international reference standard. The δ values of each standard have been defined as $0\ ^{0}/_{00}$ by either the National Institute of Standards and Technology (NIST) in the USA, or the International Atomic Energy Agency (IAEA) in Vienna (Kendall, 1998).

Various methods are currently in use for collection and preparation of nitrate for isotopic analysis (Silva *et al.*, 2000). One widely used method converts nitrate to ammonium by a Kjeldahl reaction (Bremner, 1965; Bremner and Edwards, 1965). The ammonium is then converted to dinitrogen gas by one of several methods: (1) direct combustion of the dried ammonium salt (Kendall and Grim, 1990); (2) steam distillation of

ammonium followed by oxidation with a hypobromite solution, and purification in a copper/copper oxide furnace (Bremner, 1965; Bremner and Edwards, 1965); or (3) distillation followed by collection of ammonium on an ammonium-specific zeolite, and combustion to dinitrogen gas (Velinsky *et al.*, 1989).

Many methods for measuring ^{15}N content of surface waters with low-nitrate concentration are labor-intensive and subject to potential contamination (Downs *et al.*, 1999). The use of automated Mass Spectrometers for isotopic analysis of microgram quantities of nitrate has created the need for simpler methods of recovering inorganic forms of nitrogen from water (Khan *et al.*, 1998). Thus, improved methods of determining the ^{15}N concentration of streamwater is essential (Downs *et al.*, 1999). Recently, methods have been developed to analyze nitrate for ^{15}N, improving the ability to identify nitrate sources and transformations (Chang *et al.*, 1999). In a large number of laboratories, diffusion techniques have replaced steam distillation as the method for concentrating inorganic nitrogen prior to ^{15}N analysis by continuous flow direct combustion-mass spectrometry (Stark and Hart, 1996).

Diffusion techniques are usually chosen because they are less labor-intensive, they require less skill and training, and when disposable containers are used, cross-contamination between samples is eliminated (Stark and Hart, 1996). Diffusions have been carried out in a variety of vessels, including glass digestion jars (O'Deen and Porter, 1979), canning jars (Saghir *et al.*, 1993a,b), and plastic specimen containers (Brooks *et al.*, 1989). A technique used at the University of Illinois utilizes a 1-pint wide mouth Mason jar for quantitative determination of inorganic nitrogen in soil extracts and water,

and also permits ^{15}N analysis of this nitrogen (Saghir *et al.*, 1993a,b; Khan *et al.*, 1997; Mulvaney *et al.*, 1997).

Using diffusion techniques, samples are treated with magnesium oxide (MgO) to liberate ammonium as gaseous ammonia (Khan *et al.*, 1998). The addition of Devarda's Alloy reduces both nitrate and nitrite to ammonium (Khan *et al.*, 1998). The liberated ammonia is collected using an acidified filter that is either suspended over the sample solution (Brooks *et al.*, 1989; Lory and Russselle, 1994; Herman *et al.*, 1995; Stark and Hart, 1996), or sealed inside Teflon tape for placement within the sample (Sorenson and Jensen, 1991; Stark and Hart, 1996; Downs *et al.*, 1999; Chang *et al.*, 1999). These techniques were developed to be rapid and convenient, and to facilitate nitrogen-isotope analyses by mass spectrometry (Khan *et al.*, 1998).

Waters with low nitrate concentrations are impractical to collect because of the large volumes of sample required (Silva *et al.*, 2000). As an alternative to other methods being used, anion exchange resins have been used in recent experiments to collect and concentrate nitrate from low waters with low nitrate-concentration (Chang *et al.*, 1999; Downs *et al.*, 1999; Silva *et al.*, 2000). In a recent study it was found that the placement of a cation exchange column in front of the anion exchange column minimized clogging of the anion column by dissolved organic carbon (DOC) (Chang *et al.*, 1999).

Research Objectives

The proposed research will develop a technique to analyze ^{15}N in waters with low NO_3^--concentrations, and will be tested on field samples collected in a watershed containing a first order stream.

Hypothesis

Nitrogen concentrations can be determined in water with low naturally-occurring NO_3^- - concentrations, using ^{15}N analysis.

Objectives

1. To diffuse and recover low nitrate concentrations from large sample volumes of water.
2. To determine optimal sample volume and strength and number of potassium chloride washes that are necessary to strip nitrate from anion resins, used to concentrate the nitrate.
3. To diffuse and recover concentrated nitrate using small-sample analysis techniques based on optimal volume and strength of potassium chloride washes.

METHODOLOGY

Site Description

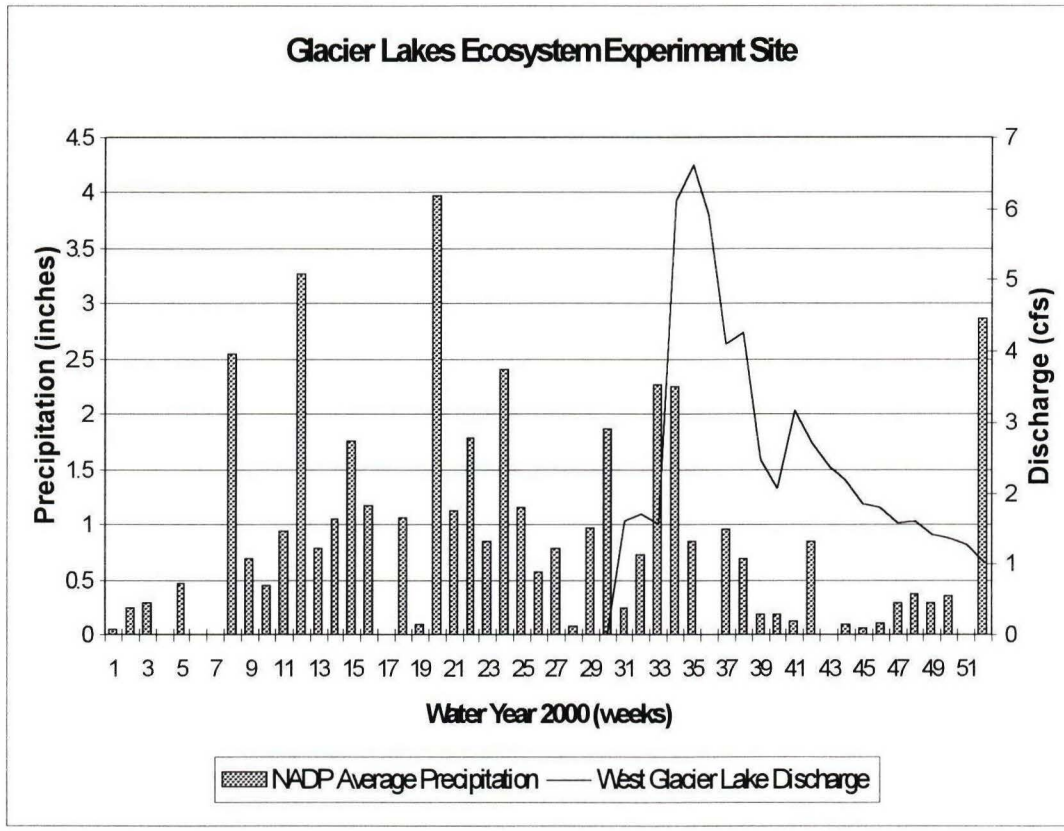
The Glacier Lakes Ecosystem Experiments Site (GLEES) is a research area located in the Snowy Range on the Medicine Bow National Forest, in south-central Wyoming. It is 55 km west of Laramie, Wyoming, and 15 km northwest of Centennial, Wyoming. It is at 41^{O} 22' 30" latitude and 106^{O} 15' 30" longitude, with elevations ranging from 3200 to 3500 m (Regan *et al.*, 1998). It was established in this wilderness-like area on the National Forest to allow construction of research structures that are not permitted on federally mandated wilderness areas (Musselman, 1994).

Bedrock in the study area is Medicine Peak Quartzite that is intruded by mafic dikes (Rochette, 1994). The quartzite bedrock is extensively fractured in most locations, with soil material filling most or all of the voids formed by these fractures (Hopper and Walthall, 1994). The high-elevation soils within the area are described by Hopper and Walthall (1994) as being primarily Cryochrepts with some Cryorthents. Low-elevation soils include Cryochrepts and Cryumbrepts. Predominant vegetation along the stream stretch is willow shrub. Away from the riparian zone, mostly coniferous forest and rock outcrops predominate.

The stream hydrology within GLEES is snow-melt dominated. The annual snowpack at GLEES is established in November and lasts into July (Sommerfeld, 1994). Mean

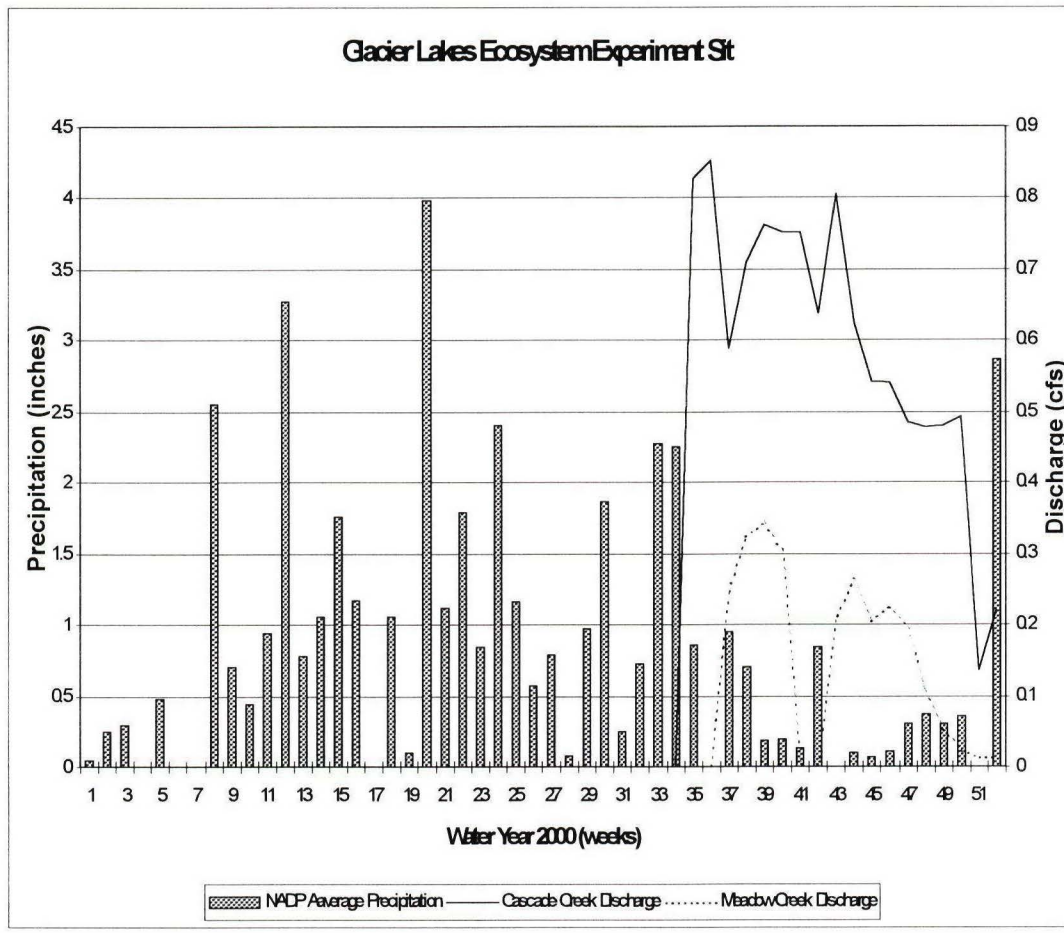
maximum snow accumulation is about 2 m at GLEES, with annual precipitation measuring about 100 cm of water (Musselman, 1994) (Figure 1).

Figure 1: GLEES: Precipitation based on weekly NADP averages, versus Discharge as recorded at West Glacier Lake Outlet



Two of four inlet streams into West Glacier Lake, Long Creek and Boulder Creek, run perennially (Finley, 1992) with input from groundwater reserves. The other two inlet streams, Cascade Creek and Meadow Creek, are fed wholly from the permanent snowfield above the lake (Musselman, 1994) (Figure 2). By late summer this source is typically dried up for Meadow Creek, while Cascade Creek flows until it freezes up in the late Fall (Figure 2).

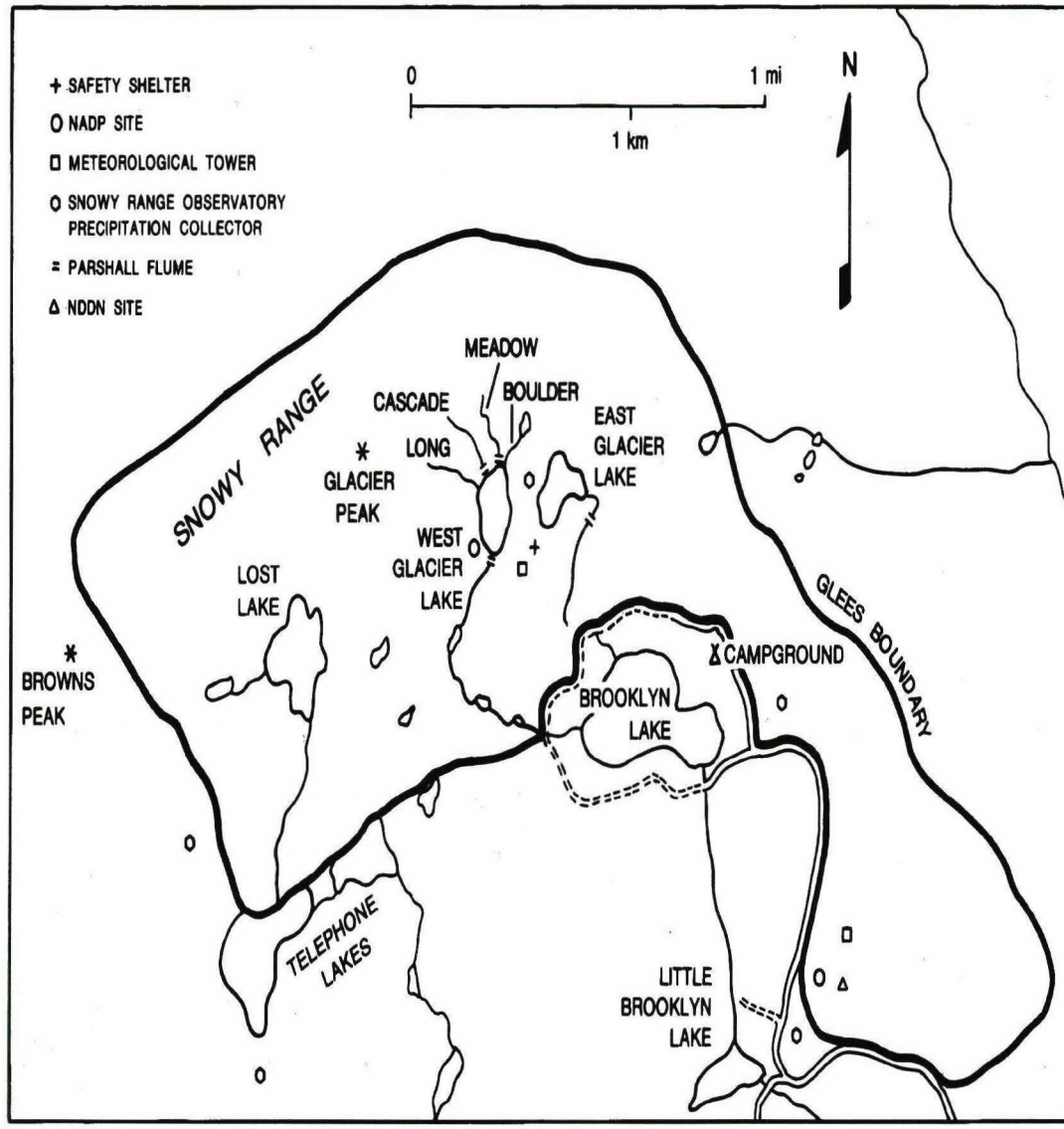
Figure 2: GLEES: Precipitation based on weekly NADP averages, versus Discharge as recorded at Cascade and Meadow Creeks



Methods

This study includes the watershed for West Glacier Lake, which comprises approximately 110 ha. With a surface area of 3.3 ha, the area feeding directly into West Glacier Lake is approximately 60.7 ha (Musselman, 1994), and includes inputs from Long, Cascade, Meadow, and Boulder Creeks. West Glacier Lake's outlet stream, which drains to the southwest through scree, wet meadow, forest, and willow thickets (Musselman, 1994) into Brooklyn Lake, is approximately 1.3 km in length (Figure 3).

Figure 3: Local area map of the Glacier Lakes Ecosystem Experiments Site (from Musselman, *et al*, July 1994).



For this study, grab samples from seven sites will be collected for determination of the ^{15}N component. Sampling sites include; each of the four inlet streams into West Glacier Lake: Long Creek (LC), Cascade Creek (CC), Meadow Creek (MC), and Boulder Creek (BC), the Parshall flume at West Glacier Lake Outlet (WGO), the point where the outlet stream flows through a culvert underneath Brooklyn Lake road (S2), and where the outlet stream flows into the west side of Brooklyn Lake (Brook-In).

Analysis for ^{15}N will be done using the Colorado State University's (CSU) Mass Spectrometer. While this machine requires a minimum nitrogen concentration of 50 μg per sample for ^{15}N analysis, an ideal sample would contain 100 μg, or more, of nitrogen per sample. To accurately test this methodology, a standard will be created using potassium nitrate (KNO_3^-) to obtain at least 100 μg of nitrogen per sample.

To ensure that field samples contain a minimum of 50 μg N per sample for ^{15}N analysis, 8 mL aliquots of each sample will be analyzed on an ion chromatograph in the USDA/FS Rocky Mountain Research Station Water Quality Lab, in Ft. Collins, CO. This analysis will determine the NO_3^- concentration, in mg/L, of each field sample. Using the NO_3^- concentration, in mg/L, the amount of N, in μg, can also be calculated. Using this calculation, the amount of sample needed can be determined to provide the requisite 50 μg N per sample for ^{15}N analysis.

Large Sample Diffusion

To determine if large amounts of water with low nitrogen content can be diffused and analyzed for ^{15}N, a Standard solution will be made up. This Standard will include 100 μg

of nitrogen per 250 mL sample, and will be made up using a 2 molar (M) KCl solution (Khan et al., 1998). The 100 μg of nitrogen per sample will be obtained using a calculated amount of KNO_3.

The Formula Weight for KNO_3 is:

$K(39.0983\ \mu g) + N(14.0067\ \mu g) + O(15.9994\ \mu g)_{x3} = 101.1032\ \mu g.$

To determine the percent N of KNO_3:

$N(14.0067\ \mu g) / KNO_3(101.1032\ \mu g) = 13.85\%$ N,

so $101.1032\ \mu g\ KNO_3 = 13.85\ \mu g$ N.

To calculate the amount of KNO_3 needed to obtain 100 μg N per sample, based on a 1 L sample:

$KNO_3\ \mu g = (101.1032\ \mu g\ KNO_3 / 13.85\ \mu g\ N)\ 100\ \mu g$ N,

or $729.987\ \mu g\ KNO_3$ for 100 μg N.

For a 250 mL sample of Standard:

$0.25\ L \times 729.987\ \mu g\ KNO_3 = 182.5\ \mu g\ KNO_3 / L$

Converting μg to mg: $182.5 \times 10^{-3} = 0.1825\ mg\ KNO_3 / L.$

The Formula Weight for KCl is:

$K(39.0983\ g) + Cl(35.4527\ g) = 74.551\ g.$

For a 2 M solution, this amount needs to be doubled to 149.102 g / L.

For a 250 mL sample: $149.102\ g \times 0.25 = 37.28$ g KCl is needed.

Sample solutions are made up using 37.28 g KCL, 0.183 mg KNO_3, and enough DI water to make up 250 mL.

To reduce nitrate and any nitrite present to ammonium, 0.4 grams of Devarda's Alloy will be mixed into the solution (Stark and Hart, 1996; Khan et al., 1998; Downs et al., 1999). Added to this solution will be 3 μg of MgO per mL of solution to liberate ammonium as gaseous ammonia (Khan et al., 1998). After capping the diffusion chamber, the solution will be swirled to obtain complete mixing.

As quickly as possible after the jar has been capped, two 7 mm disks cut from a Whatman No. 41 filter with a single-hole hole punch will be acidified by pipetting 5 μg of 2.5 M potassium bisulfate ($KHSO_4$) onto them.

The Formula Weight for $KHSO_4$ is:

$$K(39.0983\ g) + H(1.0079\ g) + S(32.066\ g) + O(15.9994)_{x4} = 120.17\ g.$$

For a 2.5 M solution, this amount needs to be multiplied by 2.5 for 300.426 g / L.

The filters will be then be wrapped in a piece of 12.5 mm by 7 cm long Teflon tape (Stark & Hart, 1996). A small test tube will be used to press on the tape around the disks to seal them in from moisture. Once the disks are acidified, they will be immediately placed into the diffusion chamber with the solution.

The Teflon tape is used to allow the ammonium to diffuse onto the acidified filter disks. By sealing the disks with the small test tube, the disks are protected from becoming saturated by the diffusion mixture while still allowing the collection of the ammonium

(Stark and Hart, 1996). The cap will be screwed on tightly and the jar inverted to reduce the possibility of leakage. The diffusion jar will be swirled at least once every three days to fully mix the solution in order to capture as much ammonium as possible (Stark & Hart, 1996). The diffusion process is lengthy, taking three to four weeks to capture all the ammonium in the sample (several iterations using the Standard, with 100 μg of nitrogen per 250 mL sample, will be run to determine the optimum diffusion time with maximum recovery of ammonium).

After diffusion is complete, the disks will be carefully retrieved from the tape using tweezers and a dissecting needle. These will both be carefully acid-washed, using hydrochloric acid (HCl), and rinsed with deionized water three times to minimize outside contamination. The disks will then be placed onto a thin acid-washed/DI rinsed wire and placed into a desiccator with a liberal quantity of sulfuric acid (H_2SO_4) in the bottom to remove the moisture (Stark and Hart, 1996). The disks will be allowed to dry completely (about one week). After carefully removing each wire containing the disks with gloved hands, the disks will be pushed off the end of the wire with a dissection needle. It is extremely important to ensure that the entire disk and any crystals that may have formed during the drying process are removed into a tin boat for analysis in the Mass Spectrometer (Heather Rueth, Pers. Comm.). The tin boats will be carefully folded over the disks in thirds, lengthwise. The tin will then be folded again, in thirds, from end to end. The last step will be to squeeze the two ends together, forming a small pellet, at which

time the sample will be stored in an airtight container until it is ready to be analyzed in the Mass Spectrometer.

Ion Exchange

A second experiment will be run using anion resins to concentrate the nitrogen in each sample prior to diffusing them. By concentrating the nitrogen into a smaller volume, it is expected that the amount of time needed to fully diffuse each sample will be greatly reduced.

For this experiment a Standard, using KNO_3 and DI water, will be made up in a 20 L carboy to obtain a concentration of 100 μg of nitrogen per liter.

To calculate the amount of KNO_3 needed to obtain 100 μg N per 1 L sample:

KNO_3 μg = (101.1032 μg KNO_3 / 13.85 μg N) 100 μg N,

or 729.987 μg KNO_3 for 100 μg N.

For 20 L of Standard:

20 L x 729.987 μg KNO_3 = 14,599.74 μg KNO_3

Converting μg to g: 14,599.74 x 10^{-6} = 0.0146 g KNO_3 / 20 L.

Added to this solution in a 1-liter bottle, will be one 1.25 mL scoop of IONAC ASB-1P (OH) hydroxide anion resin. The bottles will then be placed onto a platform shaker to

assure that the sample is continuously mixed with the anion resin. Several iterations will be run to determine the optimum exposure time to the resins. Results will be determined using ion chromatography for recovery of nitrate.

After the bottles are removed from the shaker, the sample solution will be filtered off using a Whatman No. 1 filter, leaving only the anion resins for processing. The resins will then be washed with a 2 M KCl solution to remove the nitrate anions from the exchange sites. Several iterations of this will be run as well, using both 2 M KCl and 4 M KCl in varying amounts of 3 mL washes to determine which combination results in the highest recovery of nitrate. Because the resins will be washed with highly concentrated potassium chloide, aliquots of eluate will be run on a LACHAT to determine recovery of nitrate. The eluate will then be placed into a 60 mL plastic specimen jar and diffused as described above.

Small Sample Diffusion

In a third experiment, instead of washing the anion resins down several times with small amounts of KCl, the collected resins will carefully be scraped off the filter paper with an acid-washed spatula, placed into a scintillation vial with an amount of 4 M KCl, and put onto the platform shaker for 24 hours, to ensure continuous mixing. It is expected that using this higher concentration of KCl will strip the nitrate from the standard solution more quickly than using a 2 M KCl solution.

The resins will then be filtered once again, through a Whatman No. 1 filter, with the eluate being collected for diffusion. Iterations of this experiment will include soaking the resin in different amounts of 4 M KCl to determine the best recovery of nitrate. Results will be measured using the LACHAT.

It is assumed that higher recoveries of nitrate will result in more accurate measurements of the ^{15}N isotope of low nitrate-concentration waters. Through analyzing the isotopic concentrations of headwater streams, we should be able to better tell where the nitrate is coming from in the streamwater. A more enriched sample may presumably result from anthropogenic causes, while a less enriched sample, closer to the defined natural abundance of ^{15}N equaling zero, would show concentrations of nitrogen resulting from naturally occurring nitrogen-fixing sources.

Sampling

Prior to the onset of spring snowmelt runoff, in May 2000, the Parshall flumes at West Glacier Lake Outlet and Cascade and Meadow Creeks were dug out to access their respective streams. The culvert at Brooklyn Lake road was also dug out to access that portion of the stream, for sampling.

During the first week of May, only Cascade Creek and the stream running through the culvert at Brooklyn Lake road had cleared of ice and were running. By the beginning of the second week in May, West Glacier lake outlet and Meadow Creek had also opened up

and begun to flow. During the first week of June, Boulder Creek and the inlet to Brooklyn Lake were flowing, with Long Creek opening up by the second week in June. Each of the sites were sampled weekly after they were open and flowing (Table 1).

Table 1: Grab sample nitrate concentrations determined using ion chromatography.

Sample	Day	Nitrate Concentrations : mg / L						
		LC	CC	MC	BC	WGO	S2	Brook-In
2-May-00	123		5.920				0.589	
9-May-00	130		3.722	2.473		0.976	0.633	
12-May-00	133					0.128		
16-May-00	137		3.025	1.837		1.138	0.642	
23-May-00	144		1.582	1.330		0.958	0.509	
30-May-00	151		1.408	0.715		0.812	0.561	
6-Jun-00	158		1.266	0.726	0.503	0.685	0.244	0.248
13-Jun-00	165	0.814	1.104	0.585	0.531	0.209	0.129	0.116
20-Jun-00	172	0.757	0.910	0.621	0.518	0.092	0.058	0.151
27-Jun-00	179	0.540	0.628	0.479	0.462	0.000	0.051	0.041
4-Jul-00	186	0.553	0.548	0.398	0.349	0.031	0.038	0.029
11-Jul-00	193	0.539	0.449	0.394	0.275	0.041	0.036	0.029
18-Jul-00	200	0.321	0.462	0.410	0.240	0.017	0.026	0.043
25-Jul-00	207	0.439	0.362	0.296	0.089	0.037	0.040	0.038
1-Aug-00	214	0.590	0.291	0.224	0.082	0.048	0.000	0.000
8-Aug-00	221	0.623	0.409	0.217	0.051	0.023	0.052	0.032
15-Aug-00	228	0.668	0.388	0.175	0.093	0.100	0.135	0.111
22-Aug-00	235	0.725	0.378	0.153	0.062	0.086	0.119	0.000
29-Aug-00	242	0.750	0.345	0.216	0.088	0.061	0.127	0.119
5-Sep-00	249	0.649	0.233	0.193	0.026	0.221	0.000	0.000
11-Sep-00	255	0.647	0.278	0.233	0.000	0.043	0.000	0.013
19-Sep-00-Sep-00		664	0.455	0.198	0.000	0.000	0.056	0.180
26-Sep-00	270	0.948	0.867	0.340	0.264	0.018	0.000	0.024

Initially, one liter samples were taken at each site. These samples were stored at 4° C in the Water Quality Lab at CSU. Prior to storage, an 8 mL aliquot was separated from the sample to be analyzed at the USDA/FS Rocky Mountain Research Station Water Quality Lab. Using ion chromatography, anion/cation analysis was performed to determine the concentration of nitrate in each source. Using this analysis, sample sizes for each site were calculated to meet the minimum requirement of 50 μg of nitrogen per sample needed to analyze these sample waters with mass spectrometry. Sample sizes were calculated by multiplying each nitrate concentration by 0.2258, to determine nitrogen concentrations, and then by 1000, to convert mg/L to μg/L (Table 2).

Table 2: Grab sample nitrogen concentrations (μg/L), and the calculated sample size needed to meet the 50 μg/Sample requirement for analysis on Mass Spectrometer.

Date of	Julian	Long Creek		Cascade Creek		Meadow Creek		Boulder Creek	
Sample	Date	N (μg / L)	Sample (L)	N (μg / L)	Sample (L)	N (μg / L)	Sample (L)	N (μg / L)	Sample (L)
2-May-00	123			1336.78	0.04				
9-May-00	130			840.34	0.06	558.29	0.09		
16-May-00	137			682.95	0.07	414.88	0.12		
23-May-00	144			357.31	0.14	300.20	0.17		
30-May-00	151			317.97	0.16	161.49	0.31		
6-Jun-00	158			285.86	0.17	163.93	0.31	113.58	0.44
13-Jun-00	165	183.80	0.27	249.28	0.20	132.09	0.38	119.90	0.42
20-Jun-00	172	170.93	0.29	205.48	0.24	140.22	0.36	116.96	0.43
27-Jun-00	179	121.93	0.41	141.80	0.35	108.16	0.46	104.32	0.48
4-Jul-00	186	124.87	0.40	123.74	0.40	89.87	0.56	78.80	0.63
11-Jul-00	193	121.71	0.41	101.38	0.49	88.97	0.56	62.10	0.81
18-Jul-00	200	72.48	0.69	104.32	0.48	92.58	0.54	54.19	0.92
25-Jul-00	207	99.13	0.50	81.65	0.61	66.81	0.75	20.14	2.48
1-Aug-00	214	133.22	0.38	65.71	0.76	50.58	0.99	18.52	2.70
8-Aug-00	221	140.67	0.36	92.35	0.54	49.00	1.02	11.52	4.34
15-Aug-00	228	150.83	0.33	87.61	0.57	39.52	1.27	21.00	2.38
22-Aug-00	235	163.71	0.31	85.35	0.59	34.55	1.45	14.00	3.57
29-Aug-00	242	169.35	0.30	77.90	0.64	48.77	1.03	19.87	2.52
5-Sep-00	249	146.54	0.34	52.61	0.95	43.58	1.15	5.87	8.52
11-Sep-00	255	146.09	0.34	62.77	0.80	52.61	0.95	0.00	0.00
19-Sep-00	263	149.93	0.33	102.74	0.49	44.71	1.12	0.00	0.00
26-Sep-00	270	214.06	0.23	195.77	0.26	76.77	0.65	59.61	0.84

Date of	Julian	WGO		S2		Brook-In	
Sample	Date	N (μg / L)	Sample (L)	N (μg / L)	Sample (L)	N (μg / L)	Sample (L)
2-May-00	123			133.09	0.38		
9-May-00	130	220.36	0.23	142.86	0.35		
16-May-00	137	256.92	0.19	144.92	0.35		
23-May-00	144	216.36	0.23	115.02	0.43		
30-May-00	151	183.39	0.27	126.63	0.39		
6-Jun-00	158	154.67	0.32	55.10	0.91	56.00	0.89
13-Jun-00	165	47.19	1.06	29.13	1.72	26.19	1.91
20-Jun-00	172	20.77	2.41	13.10	3.82	34.10	1.47
27-Jun-00	179	0.00	0.00	11.52	4.34	9.26	5.40
4-Jul-00	186	7.00	7.14	8.58	5.83	6.55	7.64
11-Jul-00	193	9.26	5.40	8.13	6.15	6.55	7.64
18-Jul-00	200	3.84	13.03	5.87	8.52	9.71	5.15
25-Jul-00	207	8.44	5.92	8.94	5.59	8.58	5.83
1-Aug-00	214	10.84	4.61	0.00	0.00	0.00	0.00
8-Aug-00	221	5.19	9.63	11.74	4.26	7.23	6.92
15-Aug-00	228	22.58	2.21	30.48	1.64	25.06	1.99
22-Aug-00	235	19.42	2.57	26.87	1.86	0.00	0.00
29-Aug-00	242	13.77	3.63	28.68	1.74	26.87	1.86
5-Sep-00	249	49.90	1.00	0.00	0.00	0.00	0.00
11-Sep-00	255	9.71	5.15	0.00	0.00	2.94	17.03
19-Sep-00	263	0.00	0.00	12.64	3.95	40.64	1.23
26-Sep-00	270	4.06	12.30	0.00	0.00	5.42	9.23

RESULTS

Cascade Creek

Because of a Parshall flume approximately 5 m upstream from where it flows into West Glacier Lake, Cascade Creek was the first inlet stream capable of being sampled. It had high concentrations of nitrogen at the beginning of run-off, on 2 May, with 1336.78 μg/L. Concentrations dropped steadily thereafter but remained above 100 μg/L until 25 July. With fluctuations, levels remained above 50 μg/L during the entire collection period. While concentrations dropped to levels as low as 52. 61 μg/L, on 5 September, they rose again and were back up to 195.77 μg/L by the end of the sampling period on 26 September.

Meadow Creek

While Meadow Creek began to flow on 9 May, one week after Cascade Creek, its nitrogen concentrations were lower than those found in Cascade Creek. The nitrogen concentration in Meadow Creek stayed above 50 μg/L until 8 August, when concentrations were 49 μg/L. Over the next two weeks, concentrations were as low as 34.55 μg/L. On 11 September, 3 weeks later, concentrations were at 52.61 μg/L. The following week, on 19 September, concentrations were at 44.71 μg/L. One week later, at

the end of the sampling period, concentrations were 76.77 μg/L.

Boulder and Long Creeks both enter West Glacier Lake through extensive boulder fields without Parshall flumes to measure flow. Both lakes are more difficult to sample over time, as flow can drop to levels that are unable to be reached through the boulders.

Boulder Creek

Boulder Creek was able to be sampled on 6 June, one week before Long Creek. With a concentration of 113.58 μg/L on 6 June, concentrations were at 20.14 μg/L on 25 July. Concentration were measured at 0.00 μg/L on 11 September, where they remained for two weeks. On 26 September, the last sampling date, concentrations were 59.61 μg/L.

Long Creek

Long Creek runs through a bigger boulder field than Boulder Creek and was able to be sampled one week after Boulder Creek. It also runs on the surface, at one point, where it can more easily be sampled. On 13 June concentrations were 183.80 μg/L. By 18 July concentrations were 72.48 μg/L. One week later, on 25 July, concentrations were 99.13 μg/L, with concentrations being measured above 100 μg/L for the rest of the sampling period.

West Glacier Lake Outlet

At West Glacier Lake outlet, levels stayed significantly above the 50 μg/L minimum until they dropped to 47.19 50 μg/L on 13 June. Over a two week period, this level dropped to zero. While concentrations did rise above zero again, they remained below 11 μg/L for six weeks. After dropping from 10.84 μg/L on 1 August, to 5.19 μg/L on 8 August, levels rose once again up to 49.9 μg/L on 5 September. Then, again within a two week period, levels dropped back to zero. On 26 September, at the end of the sampling period, concentrations rose back up to 4.06 μg/L.

S2

Downstream, at S2, nitrogen levels started off lower than they were at West Glacier Lake outlet, and dropped to levels lower than 50 μg/L earlier. While concentrations dropped to zero on 1 August, they rose up again to 30.48 μg/L on 15 August, two weeks later. By early September concentrations were at zero once more. After rising back up to 12.64 μg/L on 19 September, concentrations were again at zero at the end of the sampling period on 26 September.

Brook-In

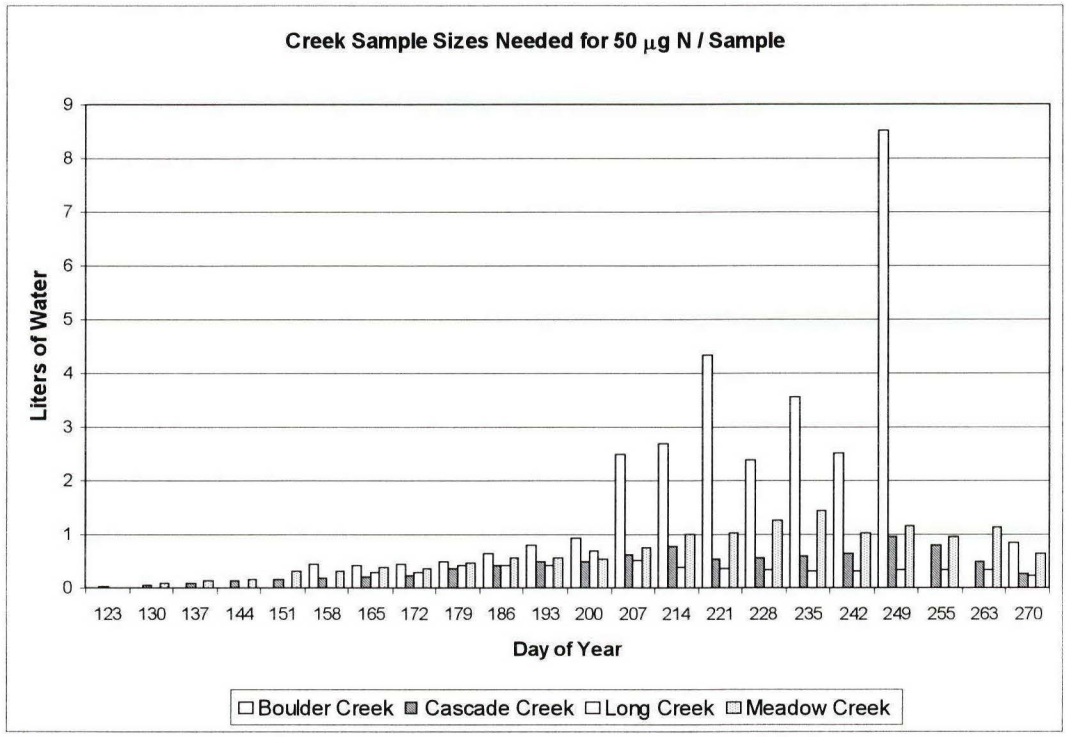
The inlet stream into Brooklyn Lake remained ice covered until 6 June, 2000. When samples were finally collected concentrations were only 56 μg/L. With some slight

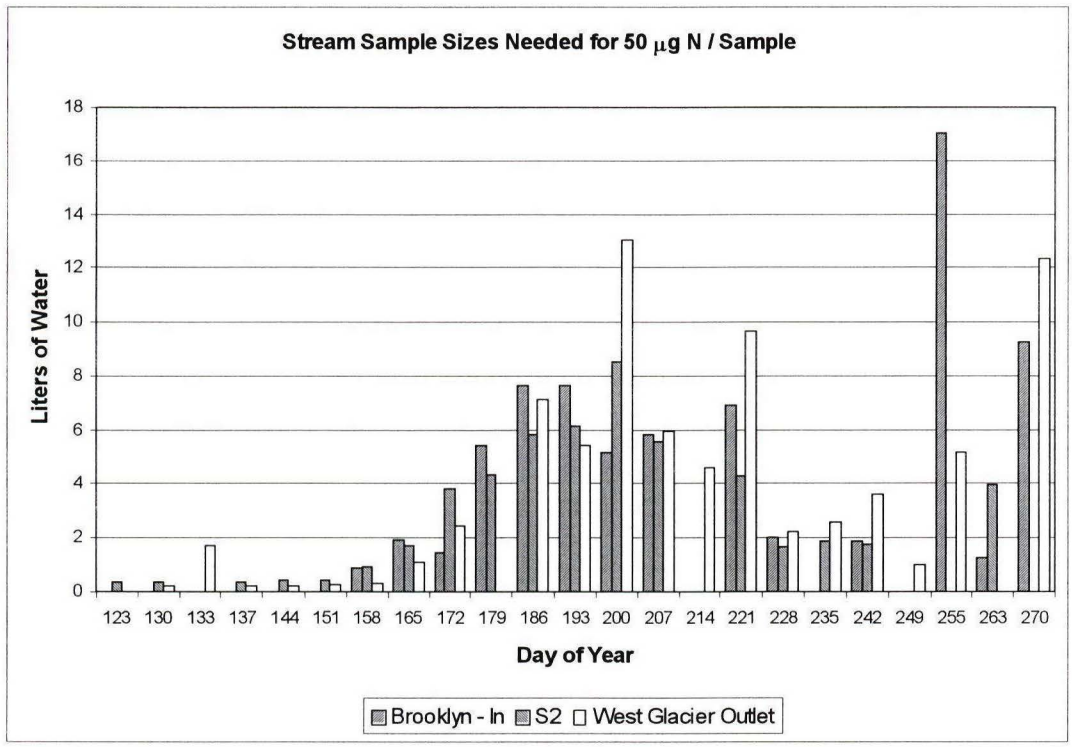
fluctuations, concentrations dropped to zero by 1 August. After increasing for two weeks, to 25.06 μg/L on 15 August, levels were again at zero on 22 August. The following week, on 29 August, concentrations were back up to 26.87 μg/L, dropping to zero yet once again by 5 September. Concentrations then rose to 2.94 μg/L on 11 September, 40.64 μg/L on 19 September, and back down to 5.42 μg/L on 26 September.

Mass Spectrometer Results

Because the Mass Spectrometer requires at least 50 μg/sample to measure a concentration, sample sizes are determined by dividing 50 μg by the calculated nitrogen concentration to calculate the number of liters of sample needed. As concentrations go down, large sample volumes are needed to meet these minimum requirements (Figure 4).

Figure 4: Sample sizes needed for 50 μg N/Sample





Large Sample Diffusions

To process these samples for analysis on the Mass Spectrometer, diffusion techniques were used to collect the nitrogen on acidified filter paper disks. To determine if lab techniques would work on these large sample sizes, diffusions were run using 250 mL of sample containing 100 μg/L of nitrogen derived from potassium nitrate mixed with deionized water.

To determine the optimum time for the diffusion of 250 mL samples, 12 samples were initially diffused over different time periods: three samples each were diffused for 10 days, 19 days, 28 days, and 38 days (Table 3).

Table 3: 250 mL Diffusion: 1st run

Sample #		Volume	Diffusion Time	TCD Area	mg N	μg N	Ave. Recovery
1	Standard	250 mL	10 days	3.5431	0.122	121.924	114.64
2	Standard	250 mL	10 days	3.2319	0.111	110.790	
3	Standard	250 mL	10 days	3.2437	0.111	111.213	
4	Standard	250 mL	19 days	3.807	0.131	131.364	136.47
5	Standard	250 mL	19 days	4.0641	0.141	140.562	
6	Standard	250 mL	19 days	3.9778	0.137	137.475	
7	Standard	250 mL	28 days	3.925	0.136	135.586	139.13
6	Standard	250 mL	28 days				
9	Standard	250 mL	28 days	4.1232	0.143	142.676	
10	Standard	250 mL	38 days	4.1996	0.145	145.410	143.40
11	Lost Sample	250 mL	38 days				
12	Standard	250 mL	38 days	4.087	0.141	141.381	

The TCD area is measured using a Mass Spectrometer. Based on readings using specific standards, a correlation curve is defined which, when the TCD area is factored in, produces a mass weight, in milligrams, for the nitrogen present in the sample. For this first run, the correlation equation for the Mass Spectrometer was: $y = (x - 0.135) / 27.95276$. Multiplying this mass by 1000 gives a mass for nitrogen in micrograms.

As can be seen in Table 3, the amount of nitrogen increased over time with each set of diffusions. While all of the measurements exceeded the initial 100 μg of nitrogen used in the standard solution, actual concentrations can't be determined since no blanks were run. Samples 8 and 11 were both lost when they were injected into the Mass Spectrometer together.

Because no blanks were run on the first set of diffusions, a second set was run using both blanks and a set of non-diffused disks. This run was also processed using 250 mL of standard spiked with 100 μg of nitrogen using potassium nitrate. Blanks were diffused with no standard being added. For the non-diffused samples, 10 μg of the potassium nitrate solution containing 100 μg of nitrogen was added directly to the disks. These disks were then dried, and analyzed on the Mass Spectrometer, without going through the diffusion process. The correlation curve used to determine the amount of nitrogen in each sample for this run was : $y = 349.59 x + 1.3591$.

While the amount of nitrogen in the diffused samples went down between the 14 day diffusions and the 21 day diffusions, it increased between the 21 day diffusions and the 28 day diffusions. There was one blank run for each of the tested time periods. All three

values for the blanks show between 18 % and 19 % recovery of the known amount of nitrogen added to the sample solution. When the values for the blanks are subtracted from the values for the samples, recovery of nitrogen appears to be between 84% and 91% for the 14 day diffusions, about 81 to 82% for the 21 day diffusions, and approximately 96% for the 28 day diffusions. The non-diffused samples show only about a 50% recovery (Table 4).

Table 4: 250 mL Diffusion: 2nd run

Sample #		Volume	Diffusion Time	TCD Area	mg N	µg N	Blank Corrected	Ave. Recovery
1A	Standard	250	0 days	25.0574	0.052	51.870		49.87
1B	Standard	250	0 days	23.5366	0.048	47.681		
1C	Standard	250	0 days	24.4039	0.050	50.070		
2A	Standard	250	14 days	43.6289	0.103	103.019	84.16	87.16
2B	Standard	250	14 days	46.0439	0.110	109.671	90.81	
2C	Standard	250	14 days	44.4847	0.105	105.377	86.51	
2D	Blank	250	14 days	13.0736	0.019	18.864		
3A	Standard	250	21 days	42.3018	0.099	99.364	80.76	81.40
3B	Standard	250	21 days	42.7103	0.100	100.489	81.89	
3C	Standard	250	21 days	42.5926	0.100	100.165	81.56	
3D	Blank	250	21 days	12.9788	0.019	18.603		
4A	Standard	250	28 days	47.8059	0.115	114.524	96.05	96.28
4B	Standard	250	28 days	48.1327	0.115	115.424	96.95	
4C	Standard	250	28 days	47.7305	0.114	114.316	95.84	
4D	Blank	250	28 days	12.932	0.018	18.474		

With concentrations dropping between 14 and 21 days, then rising up again at 28 days, results indicate that more than 28 days are needed to recover the nitrogen through diffusion. Because of this, a decision was made to use anion resins in an ion exchange process to first concentrate the nitrogen in the sample prior to collecting it using the diffusion process. By using ion exchange to concentrate the nitrogen in the sample, it was also considered that the amount of eluate to be diffused would be much less than the 250 mL used in the first two experiments.

Ion Exchange

Ion exchange uses anion resins, which are resins with a positive charge that concentrate negatively charged anions onto available exchange site. This means that nitrate in the sample adsorbs onto available exchange sites on the resin and is held in place until another anion, with higher ionic bonding energy, is introduced to replaced it. To strip the nitrate off the resins, a solution of potassium chloride was used. In designing an experiment using ion exchange, it is necessary to determine how much, and what strength potassium chloride must be used to optimally strip the nitrate off the resins.

The first run using ion exchange used a standard solution made up with potassium nitrate and deionized water to provide 100 µg/L of nitrogen. The resin was measured out using a ¼ teaspoon scoop, which gave a measure of 1.25 mL of resin. Potassium chloride was measured out in both 2 molar and 4 molar strengths. A pipettor was used to measure out 3 mL washes of the potassium chloride.

Samples were run in series of threes: three samples using one 3 mL wash of 2 molar potassium chloride, three samples using two 3 mL washes of 2 molar potassium chloride, and three samples using two 3 mL washes of 4 molar potassium chloride. A full set of nine samples

was put on a table shaker for 4, 8, and 24 hours to determine the optimum time for ion exchange with the resins (Table 5).

Table 5: Ion Exchange : 1st Run

Sample #		On Shaker	KCl	Wash	mg NO3 - N	mg N	Blank Corrected	Ave. Recovery
101	Standard	4 hours	2 M	3 mL	Lost Sample			
102	Standard	4 hours	2 M	3 mL	Lost Sample			
103	Standard	4 hours	2 M	3 mL	Lost Sample			
131	Blank	4 hours	2 M	3 mL	Lost Sample			
104	Standard	4 hours	2 M	6 mL	1.062	6.372		5.94
105	Standard	4 hours	2 M	6 mL	Lost Sample			
106	Standard	4 hours	2 M	6 mL	0.917	5.502		
132	Blank	4 hours	2 M	6 mL	Lost Sample			
107	Standard	4 hours	4 M	6 mL	1.311	7.866	6.64	7.68
108	Standard	4 hours	4 M	6 mL	1.656	9.936	8.71	
109	Standard	4 hours	4 M	6 mL	Lost Sample			
110	Blank	4 hours	4 M	6 mL	0.204	1.224		1.22
111	Standard	8 hours	2 M	3 mL	Lost Sample			
112	Standard	8 hours	2 M	3 mL	Lost Sample			
113	Standard	8 hours	2 M	3 mL	Lost Sample			
114	Standard	8 hours	2 M	6 mL	0.901	5.406	4.67	4.56
115	Standard	8 hours	2 M	6 mL	0.785	4.710	3.98	
116	Standard	8 hours	2 M	6 mL	0.958	5.748	5.02	
120	Blank	8 hours	2 M	6 mL	0.122	0.732		0.73
117	Standard	8 hours	4 M	6 mL	1.173	7.038		7.93
118	Standard	8 hours	4 M	6 mL	1.341	8.046		
119	Standard	8 hours	4 M	6 mL	1.451	8.706		
121	Standard	24 hours	2 M	3 mL	Lost Sample			
122	Standard	24 hours	2 M	3 mL	Lost Sample			
123	Standard	24 hours	2 M	3 mL	Lost Sample			
130	Blank	24 hours	2 M	3 mL	Lost Sample			
124	Standard	24 hours	2 M	6 mL	2.098	12.588		10.67
125	Standard	24 hour.016	1.512			9.072		
126	Standard	24 hours	2 M	6 mL	1.726	10.356		
127	Standard	24 hours	4 M	6 mL	2.421	14.526		16.43
128	Standard	24 hours	4 M	6 mL	Lost Sample			
129	Standard	24 hours	4 M	6 mL	3.056	18.336		

It was found that the pipettor was producing 1.75 mL washes instead of 3 mL washes. As a result, several of the samples did not produce enough eluate to be analyzed on the LACHAT. Because of this, a second run was set up, also run in series of three.

For this experiment 24 samples were set up: using both three 3 mL 4 molar potassium chloride washes, and five 3 mL 4 molar potassium chloride washes, three samples were run using one scoop of resin, and three samples were run using two scoops of resin. It was assumed that the stronger 4 molar potassium chloride would more effectively strip the nitrate from the anion resins. Blanks were also run for each of the four situations. A total of 16 samples was put on the shaker for 6 hours, as well as for 24 hours (Table 6).

Table 6: Ion Exchange : 2nd Run

Sample #		On Shaker	Resin	KCl	Wash	mg NO3 - N	mg N	Blank Corrected	Ave. Recovery
133	Standard	6 hours	1 scoop	4 M	9 mL	3.197	28.773	23.74	21.17
134	Standard	6 hours	1 scoop	4 M	9 mL	2.579	23.211	18.18	
135	Standard	6 hours	1 scoop	4 M	9 mL	2.959	26.631	21.60	
145	Blank	6 hours	1 scoop	4 M	9 mL	0.559	5.031		
136	Standard	6 hours	2 scoops	4 M	9 mL	3.698	33.282	25.44	19.29
137	Standard	6 hours	2 scoops	4 M	9 mL	2.313	20.817	12.98	
138	Standard	6 hours	2 scoops	4 M	9 mL	3.032	27.288	19.45	
146	Blank	6 hours	2 scoops	4 M	9 mL	0.871	7.839		
139	Standard	6 hours	1 scoop	4 M	15 mL	2.325	34.875	27.84	31.26
140	Standard	6 hours	1 scoop	4 M	15 mL	2.635	39.525	32.49	
141	Standard	6 hours	1 scoop	4 M	15 mL	2.699	40.485	33.45	
147	Blank	6 hours	1 scoop	4 M	15 mL	0.469	7.035		
142	Standard	6 hours	2 scoops	4 M	15 mL	3.336	50.040	37.71	42.46
143	Standard	6 hours	2 scoops	4 M	15 mL	3.237	48.555	36.23	
144	Standard	6 hours	2 scoops	4 M	15 mL	4.385	65.775	53.45	
148	Blank	6 hours	2 scoops	4 M	15 mL	0.822	12.330		
149	Standard	24 hours	1 scoop	4 M	9 mL	8.048	72.432	67.11	66.79
150	Standard	24 hours	1 scoop	4 M	9 mL	8.839	79.551	74.23	
151	Standard	24 hours	1 scoop	4 M	9 mL	7.148	64.332	59.01	
161	Blank	24 hours	1 scoop	4 M	9 mL	0.591	5.319		
152	Standard	24 hours	2 scoops	4 M	9 mL	5.419	48.771	42.68	37.59
153	Standard	24 hours	2 scoops	4 M	9 mL	4.183	37.647	31.55	
154	Standard	24 hours	2 scoops	4 M	9 mL	4.958	44.622	38.53	
162	Blank	24 hours	2 scoops	4 M	9 mL	0.677	6.093		
155	Standard	24 hours	1 scoop	4 M	15 mL	6.415	96.225	89.03	80.04
156	Standard	24 hours	1 scoop	4 M	15 mL	6.400	96.000	88.80	
157	Standard	24 hours	1 scoop	4 M	15 mL	4.632	69.480	62.28	
163s			1 scoop	4 M	15 mL	0.480	7.200	62.28	
158	Standard	24 hours	2 scoops	4 M	15 mL	4.396	65.940	51.89	48.05
159	Standard	24 hours	2 scoops	4 M	15 mL	2.962	44.430	30.38	
160	Standard	24 hours	2 scoops	4 M	15 mL	5.063	75.945	61.89	
164	Blank	24 hours	2 scoops	4 M	15 mL	0.937	14.055		

For those samples on the shaker for 6 hours, there was no real difference between the use of one scoop or two scoops of resin. One scoop of resin with three 3 mL washes recovered an average of 26.21 μg of nitrogen, while two scoops of resin recovered an average of 27.13 μg of nitrogen. Using five 3 mL washes, one scoop of resin recovered an average 38.3 μg of nitrogen, while two scoops recovered 54.79 μg of nitrogen, which is significantly greater than using only three washes. Reducing these amounts by the recovery measured in the blanks results in values of 21.17 μg, 19.29 μg, 31.26 μg, and 42.46 μg of nitrogen, respectively.

For those samples on the shaker for 24 hours, there was a significant difference between the use of one scoop and two scoops of resin. The use of one scoop of resin, with both three 3 mL washes and five 3 mL washes, resulted in a much higher recovery than when two scoops of resin was used. While in both instances the use of two scoops of resin resulted in less than 50% recovery, with the blanks subtracted out, one scoop of resin resulted in a 67% recovery, with three 3 mL washes, and 80% recovery with five 3 mL washes of 4 molar potassium chloride. To further increase recovery, it was decided that instead of performing several 3 mL washes with the resins, it would be more efficient to soak the resins in potassium chloride to increase exposure time.

Small Sample Diffusion

In a third experiment, one scoop of resin was placed into a 1 L sample to concentrate the nitrogen. After being on the shaker for 24 hours, the resin was filtered out of the sample and placed into a scintillation vial with 10 mL of 4 molar potassium chloride. This solution was also put onto a shaker for 24 hours, after which the resin was again filtered out of

solution. This solution was then analyzed, using the LACHAT, to determine recovery (Table 7).

Table 7: Ion Exchange : 3rd Run

Sample #		On Shaker	Resin	KCl	Wash	mg NO3 - N	mg N	Blank Corrected	Ave. Recovery
168	Standard	24 hours	1 scoop	4 M	10 mL	0.592	5.920	4.18	13.56
169	Standard	24 hours	1 scoop	4 M	10 mL	1.655	16.550	14.81	
170	Standard	24 hours	1 scoop	4 M	10 mL	1.631	16.310	14.57	
171	Standard	24 hours	1 scoop	4 M	10 mL	2.083	20.830	19.09	
172	Standard	24 hours	1 scoop	4 M	10 mL	1.688	16.880	15.14	
173	Blank	24 hours	1 scoop	4 M	10 mL	0.166	1.660		1.74
174	Blank	24 hours	1 scoop	4 M	10 mL	0.149	1.490		
175	Blank	24 hours	1 scoop	4 M	10 mL	0.206	2.060		

Deionized water was used to rinse the resins to ensure capturing as much eluate as possible. However, this not only diluted the eluate, but skewed results on the LACHAT as well. The analysis resulted in very low recovery numbers. Because of this, it was necessary to rerun the experiment a fourth time.

The fourth run was essentially a repeat of the third run, except that when the resin was filtered out of the potassium chloride, it was rinsed down with additional potassium chloride. While the results were much better in this run, it appears that the additional potassium chloride also diluted the results somewhat (Table 8).

Table 8: Ion Exchange : 4th Run

Sample #		On Shaker	Resin	KCl	Wash	mg NO3 - N	mg N	Blank Corrected	Ave. Recovery
180	Blank	24 hours	1 scoop	4 M	10 mL	0.654	6.540		6.18
181	Blank	24 hours	1 scoop	4 M	10 mL	0.688	6.880		
182	Blank	24 hours	1 scoop	4 M	10 mL	0.513	5.130		
183	Standard	24 hours	1 scoop	4 M	10 mL	7.271	72.710	66.53	63.54
184	Standard	24 hours	1 scoop	4 M	10 mL	7.017	70.170	63.99	
185	Standard	24 hours	1 scoop	4 M	10 mL	7.322	73.220	67.04	
186	Standard	24 hours	1 scoop	4 M	10 mL	7.756	77.560	71.38	
187	Standard	24 hours	1 scoop	4 M	10 mL	5.497	54.970	48.79	

Running the experiment a fifth time, resins from three samples were placed into 15 mL of potassium chloride, and resins from another three samples were placed into 20 mL of potassium chloride, to optimize recovery. When the resins were filtered out of the potassium chloride, the undiluted eluate was analyzed on the LACHAT. As can be seen, the results using 15 mL of potassium chloride to strip the resins resulted in 84.33 μg of nitrogen, where the results using 20 mL of potassium chloride resulted in 81.98 μg of nitrogen (Table 9).

Table 9: Ion Exchange : 5th Run

Sample #		On Shaker	Resin	KCl	Wash	mg NO3 - N	mg N	Blank Corrected	Ave. Recovery
194	Standard	24 hours	1 scoop	4 M	15 mL	6.662	99.930	91.00	84.33
195	Standard	24 hours	1 scoop	4 M	15 mL	5.707	85.605	76.67	
196	Standard	24 hours	1 scoop	4 M	15 mL	6.284	94.260	85.33	
197	Blank	24 hours	1 scoop	4 M	15 mL	0.644	9.660		8.93
198	Blank	24 hours	1 scoop	4 M	15 mL	0.547	8.205		
199	Standard	24 hours	1 scoop	4 M	20 mL	4.126	82.520	72.81	81.98
200	Standard	24 hors	1 scoop	4 M	20 mL	4.801	96.020	86.31	
201	Standard	24 hours	1 scoop	4 M	20 mL	4.826	96.520	86.81	
202	Blank	24 hours	1 scoop	4 M	20 mL	0.438	8.760		9.71
203	Blank	24 hours	1 scoop	4 M	20 mL	0.533	10.660		

Based on these results, a diffusion was run to determine how much nitrogen could be recovered diffusing only 15 mL of eluate rather than 250 mL. Samples were obtained through ion exchange, using one scoop of resin in 1 L of standard solution containing 100 μg of nitrogen. After 24 hours on a shaker, the nitrogen collected on the resin was stripped off by soaking the resin in 15 mL of 4 molar potassium chloride for an additional 24 hours.

Using 60 mL Nalgene specimen cups, nitrogen was diffused onto acidified disks, using a mixture of Devarda's Alloy and magnesium oxide to reduce the nitrate to ammonia. The disks were dried, wrapped in tin boats and analyzed using a Mass Spectrometer. Based on several standards, the correlation equation for measuring nitrogen was:

$y = (0.0322\ x) - 0.0075$ (Table 10).

Table 10: Diffusion based on Ion Exchange

Sample #		Volume	Diffusion Time	TCD Area	mg N	µg N	Blank Corrected	Ave. Recovery
204	Standard	15 ml	2 days	2.9252	0.087	86.691	33.52	30.69
205	Standard	15 ml	2 days	2.8207	0.083	83.327	30.15	
206	Standard	15 ml	2 days	2.7666	0.082	81.585	28.41	
207	Blank	15 ml	2 days	2.8966	0.086	85.771		53.18
209	Blank	15 ml	2 days	0.87207	0.021	20.581		
210	Standard	15 ml	4 days	2.4658	0.072	71.899	52.55	45.43
211	Standard	15 ml	4 days	2.4118	0.070	70.160	50.81	
212	Standard	15 ml	4 days	1.8569	0.052	52.292	32.94	
213	Blank	15 ml	4 days	0.81457	0.019	18.729		19.35
214	Blank	15 ml	4 days	0.8532	0.020	19.973		
216	Standard	15 ml	7 days	2.6528	0.078	77.920	55.92	54.16
217	Standard	15 ml	7 days	2.5568	0.075	74.829	52.83	
218	Standard	15 ml	7 days	2.5854	0.076	75.750	53.75	
219	Blank	15 ml	7 days	0.81677	0.019	18.800		22.00
220	Blank	15 ml	7 days	1.0157	0.025	25.206		

When the diffusion chambers were put on the shaker, enough hydrogen gas was produced that sample solution was lost through the threads of the chamber. On noticing this loss, the chambers were turned right-side up to preserve the solution. It is assumed however, that as the hydrogen gas was lost, some nitrogen, in the form of ammonia, was lost as well. This would result in low recovery of the amount of nitrogen known to have been in the original standard solution.

A final ion exchange and diffusion was run, using ½ pint glass Ball jars as diffusion chambers. These jars have a gasket on the lid specifically designed to withhold high pressures. As can be seen, the results were much higher using this technique (Table 11). However, when the blanks are subtracted out, results still fall below 75%.

Table 11: Final Diffusion based on Ion Exchange

Sample #		Volume	Diffusion Time	TCD Area	mg N	µg N	Blank Corrected	Ave. Recovery
252	Standard	15 ml	2 days	2.8376	0.084	83.871	67.51	68.95
253	Standard	15 ml	2 days	2.8722	0.085	84.985	68.62	
254	Standard	15 ml	2 days	2.9372	0.087	87.078	70.72	
255	Blank	15 ml	2 days	0.72669	0.016	15.899		16.36
256	Blank	15 ml	2 days	0.75532	0.017	16.821		
257	Standard	15 ml	4 days	2.9639	0.088	87.938	72.03	72.21
258	Standard	15 ml	4 days	2.9767	0.088	88.350	72.44	
259	Standard	15 ml	4 days	2.9683	0.088	88.079	72.17	
260	Blank	15 ml	4 days	0.69722	0.015	14.950		15.91
261	Blank	15 ml	4 days	0.75695	0.017	16.874		
262	Standard	15 ml	7 days	3.1921	0.095	95.286	72.50	73.11
263	Standard	15 ml	7 days	3.2179	0.096	96.116	73.33	
264	Standard	15 ml	7 days	3.2229	0.096	96.277	73.49	
265	Blank	15 ml	7 days	0.99246	0.024	24.457		22.78
266	Blank	15 ml	7 days	0.88856	0.021	21.112		

CONCLUSION

Three experiments were run in this study to determine if the methodology used could produce samples capable of being measured for ^{15}N on a mass spectrometer. The experiment run for Large Sample Diffusions resulted in the determination that more than 28 days was needed to diffuse large samples (250 mL) to analyze nitrogen concentrations. Even at 28 days, concentrations had not leveled out to a point where it could be concluded that all the nitrogen introduced in the sample was recovered.

While the second run for the 250 mL diffusions measured an average 96% recovery at 28 days, the recovery at 21 days was lower than that at 14 days. The upward trend between 21 and 28 days is inconclusive and is not comparable to the results from the first run, as no blanks were run on the first run to correct the final results. At least one, and preferably several more iterations should have been run to verify the results of the second run. However, because of the length of time involved as well as the experiment being run on large volumes, it was reasonably decided to change the experiment to one using anion resins to concentrate the nitrogen in the samples prior to diffusing them for ^{15}N analysis.

Several iterations of the experiment using anion resins were run to determine optimal volumes and strengths pf components to most effectively strip nitrate anions from the resins. The results of these experiments was that anions were more efficiently stripped when the resins were soaked over time in a KCL wash, instead of being washed off with smaller volume washes.

Based on the results formulated by running the initial experiments using anion resins, it was determined that the resins would be allowed to concentrate the nitrogen present for 24 hours. After removing the resins and stripping the nitrogen anions off them with 4 M KCl for another 24 hours, I was able to diffuse the samples using only 15 mL of sample.

Because the amount of sample was decreased over those used in the Large Sample Diffusions, the Small Sample Diffusions were accomplished in only 7 days. However, the results are such that the recovery, corrected with the blank recoveries, shows concentrations of approximately 73%. While I was looking for recoveries in the 90 - 95 % range, these results were disappointing.

By introducing the several additional steps involved in processing the samples with anion resins, error was introduced. In addition, there were several problems encountered using different vessels as diffusion chambers. In several instances, there was leakage of the diffused sample, both in terms of volume of liquid as well as gas. It is thought that were time and materials available, additional repetitions could be run in order to reduce this variability.

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